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Berberine acts as a putative epigenetic modulator by affecting the histone code

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ABSTRACT

Berberine, an isoquinoline plant alkaloid, exhibits a wide range of biochemical and pharmacological effects. However, the precise mechanism of these bioactivities remains poorly understood. In this study, we found significant similarity between berberine and two epigenetic modulators (CG-1521 and TSA). Reverse-docking using berberine as a ligand identified lysine-*N*-methyltransferase as a putative target of berberine. These findings suggested the potential role of berberine in epigenetic modulation. The results of PCR array analysis of epigenetic chromatin modification enzymes supported our hypothesis. Furthermore, the analysis showed that enzymes involved in histone acetylation and methylation were predominantly affected by treatment with berberine. Up-regulation of histone acetyltransferase CREBBP and EP300, histone deacetylase SIRT3, histone demethylase KDM6A as well as histone methyltransferase SETD7, and down-regulation of histone acetyltransferase HDAC8, histone methyltransferase WHSC1I, WHSC1II and SMYD3, in addition to 38 genes from histone clusters 1–3 were observed in berberine-treated cells using real-time PCR. In parallel, western blotting analyses revealed that the expression of H3K4me3, H3K27me3 and H3K36me3 proteins decreased with berberine treatment. These results were further confirmed in acute myelocytic leukemia (AML) cell lines HL-60/ADR and KG1-α. Taken together, this study suggests that berberine might modulate the expression of epigenetic regulators important for many downstream pathways, resulting in the variation of its bioactivities.

1. Introduction

Chemotherapy plays an important role in the treatment of multiple myeloma (MM), acute myelocytic leukemia (AML) and other malignant hematological diseases. In recent years, combination chemotherapy and stem cell transplantation greatly increased the rate of complete remission in MM and AML. However, improvements were less pronounced in relapsed patients. The unmet need in this population may be addressed using optimal chemotherapy combination and novel agents (Brenner et al., 2008; Jiang et al., 2012; Tallman et al., 2005).

Berberine is a major botanical alkaloid extracted from the roots and bark of many plants, such as *Hydrastis canadensis*, *Cortex phellodendri* (Huangbai) and *Rhizoma coptidis* (Huanglian). Recent studies have shown that berberine induces apoptosis and exerts anti-angiogenic activity in various types of cancer cells. This mechanism is mainly mediated via the inhibition of various pro-inflammatory cytokines and pro-angiogenic factors, such as HIF, VEGF, COX-2, NO and NF-κB (Hamsa and Kuttan, 2012; Kaiser et al., 2006). Zhang et al. (2010) reported that the pro-apoptotic effects of berberine are closely associated with the down-regulation of MDM2 followed by a steady-state activation of p53 in MDM2-overexpressing chemoresistant cancer cells, which play an important role in treating refractory patients. Berberine also induces p53-independent, XIAP-mediated apoptotic cell death in p53-null leukemia cells (Liu et al., 2013).

However, how these molecular events are regulated remains obscure. Because the reported events are so diverse, berberine is considered to regulate upstream rather than downstream molecular switches. As previously reported, epigenetic changes may play an important role in the pathogenesis of multiple myeloma and other tumors (Kaiser et al., 2013). Furthermore, changes in histone methylation/acetylation may be of particular importance in the pathogenesis of MM and AML, and targeting enzymes responsible for histone post-translational

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modifications may be a novel and promising therapeutic strategy in the future (Anon, 2013; Smith et al., 2010). Our experiments independently confirmed the effect of berberine on growth suppression and cell cycle cessation and its ability to enhance ROS levels and to inhibit IL6 production in multiple myeloma cells (HY Hu, data to be published separately). Our bioinformatics analysis suggested that berberine altered epigenetic patterns in multiple myeloma U266 cells. Experimental validations support our working hypothesis that berberine may regulate the epigenetic switches that govern diverse downstream bioactivities and suppress the growth of AML cells except U266 cells. Taken together, these findings may shed new light on the mechanism underlying berberine activation of various biological events, including, but not limited to, its anti-cancer activities.

2. Materials and methods

2.1. Cell line and reagents

The multiple myeloma cell line U266 was kindly provided by Professor Jie Jin (Hematology Department of The First Affiliated Hospital, Medicine College, Zhejiang University). HL-60/ADR and KG1-α cell lines were obtained from the Institutes for Biological Sciences Cell Resource Center, Chinese Academy of Sciences, Shanghai, China. The cells were maintained in RPMI1640 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) at 37 °C in a 5% CO_2 incubator. Concentrated stock solutions of berberine (Sigma-Aldrich, USA) were diluted in DMSO (Sigma-Aldrich, USA). RNA-grade TRIzol was purchased from Invitrogen (CA, USA). The RevertAid™ First Strand cDNA Synthesis Kit and SYBR Premix Ex Taq II kit were obtained from Fermentas (USA) and Takara (Japan), respectively.

2.2. Antibodies

The primary antibodies used in this study were the following: anti-H3K4me2 polyclonal antibody (CST, #9726), anti-H3K4me3 polyclonal antibody (Abcam, #ab8580), anti-H3K27me3 monoclonal antibody (CST, #9733), anti-H3K36me3 monoclonal antibody (CST, #4909), and anti-Histone3 monoclonal antibody (CST, #3377).

2.3. RNA extraction

U266 cells were seeded in 75 cm^2 plates at a concentration of 1×10^7 cells/plate and were treated for 48 h with berberine at final concentrations of 0, 40, 80, 120 and 160 μM. In addition, the concentrations of berberine used in HL-60/ADR and KG1-α cells were 0, 11, 22, and 44 μM due to different IC50s (half maximal inhibitory concentration). RNA was extracted using TRIzol (Invitrogen, USA) according to the manufacturer's recommended protocol. The quantity and purity of RNA were determined by measuring A260 and A280 using a NanoDrop ND-1000 (NanoDrop). The integrity of the RNA was determined using 1.5% agarose gel electrophoresis.

2.4. Gene expression microarray

Total RNA was separately harvested and purified from U266 cells of the control group and berberine (120 μM)-treated group. RNA sample amplification and labeling were performed using the Amino Allyl MessageAmp™ II aRNA Amplification Kit (Ambion, Inc.) according to the manufacturer's instructions. Samples (4 μg) labeled with Cy3/Cy5 were hybridized and processed on Chipscreen (Shenzhen, China) operon human oligo microarray CSC-GE-30, which covered ~35,000 transcripts. Scanning was performed with the LuxScan™ 10 K Microarray Scanner under settings recommended by CapitalBio (Beijing, China). After removal of negative-flagged probes and data normalization using R's "limma" package, differential genes were selected based on the fold-change for functional enrichment using R programming (www.cran.r-project.org) and the Nextbio platform (www.nextbio.com). Gene set enrichment was performed using our in-house web tool, in which the main data sources were provided by MSigDB (Broad Institute), Reactome, Biocarta and Gene Ontology Consortium, in addition to the gene sets collected from literature mining.

2.5. PCR array analysis of epigenetic chromatin modification enzymes

To profile the gene expression of epigenetic chromatin modification enzymes, we employed Epigenetic Chromatin Modification Enzymes PCR Array (SABiosciences, QIAGEN). The array covers 84 genes of epigenetic factors from several families, including five housekeeping genes (B2M, HPRT1, RPL13A, GAPDH, ACTB). The PCR array was performed by Shanghai Kangchen Biotech Company (Shanghai, China). For this experiment, U266 cells were treated with 80 μM of berberine for 48 h. An RT^2 First Strand Kit (SABiosciences) was used to prepare cDNAs from the RNA samples. Next, cDNAs were added to the RT^2 qPCR Master Mix, containing SYBR Green and reference dye, and subsequently amplified for 10 min at 95 °C, 40 PCR cycles (15 s at 95 °C, 1 min at 60 °C) according to the manufacturer's recommended protocol. After the PCR reaction, B2M, RPL13A and ACTB were used as internal references for data normalization.

2.6. Growth inhibition and apoptosis assay

Cells were respectively treated with different concentrations of BBR (berberine). Antiproliferative effect was assessed by employing MTS (a soluble tetrazolium salt) assay. Cells were cultured for 20, 44, 68 and 68 h in 96-well microtiter plates with different concentrations of BBR. Then, the MTS was added and the incubation Cell Titer continued for additional 4 h. Absorbance was measured at 490 nm using a spectrophotometer (BIO-RAD 680, USA), and cell viability was determined in each group and compared with that of the untreated cells. After 48 h culture, untreated and drug-treated cells were collected, and washed twice with cold PBS (pH 6.9, each for 5 min), stained with Annexin V-FITC/PI (NanJing KeyGen Biotechnology, NanJing, China), and subjected to flow cytometry (Becton Dickinson, CA, USA) analysis of apoptosis.

2.7. Real-time PCR

Additional PCR reactions were performed to cover a few more selected genes. The cDNAs were synthesized using reverse transcription from 0.5 μg total RNAs using oligo (dT) primers. Real-Time PCR reactions were performed using the SYBR Green PCR Master Mix (Takara) on a LightCycler480 System (Roche). The primer sequences for the chromatin modification genes and house-keeping gene GAPDH are provided in Supplementary data 1.

2.8. Western blotting analysis

Berberine was added to U266 cells at final concentrations of 0, 40, 80, 120 and 160 μM for 24 h. In addition, HL-60/ADR and KG1-α cells were treated with 0, 11, 22 μM berberine for 48 h. Protein concentrations of the SDS lysates were determined using the bicinchoninic acid method (Tjabringa et al., 2003).

2.9. Statistical analyses

Each experiment was performed in triplicate and repeated at least three times. All data from these experiments were expressed as the mean ± S.E.M using SPSS13.0 software. The difference among the means of multiple groups was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey test and LSD test when equal variances were assumed, while Dunnett's T3 test and Dunnett's C test were used when equal variances were not assumed. A difference was defined as significant at $p < 0.05$.

Table 1
Results of epigenetic chromatin modification enzymes PCR array.

Family	Up-regulation	Down-regulation
DNA methyltransferase		DNMT1/3B
Histone acetyltransferase	CREBBP, EP300, HAT1, NCOA, KAT	
Histone deacetylase	HDAC5/9	HDAC2/8
Lysine *N*-methyltransferase/SET domain proteins	SETD1B/B1/B2/2/3/5/6/7/8, SMYD3[a]	
Histone lysine (K)-specific demethylase	KDM4A/5B/6B/1A	WHSC1
Histone phosphorylation	AURKC	AURKB
Histone ubiquitination	DZIP3	UBE2A/2B
Histone arginine(R) *N*-methyltransferase	PRMT2/5/6/7/8	PRMT1

The p values ($p < 0.05$) were calculated based on Student's t-test comparing replicate $2^{-\triangle\triangle Ct}$ values for each gene between the control and the treatment groups. Genes showing at least 1.5-fold change were listed.

[a] Inconsistency between PCR array RT-qPCR and microarray.

3. Results

3.1. Berberine induced gene expression changes in the epigenome

Global changes in gene expression induced by berberine in U266 cells were examined using the Chipscreen operon human oligo microarray, which covers approximately 35,000 transcripts of the human genome. Compared with the control group, 2599 genes were up-regulated and 2155 genes were down-regulated at least two-fold with berberine treatment. Gene set enrichment analysis showed that 38 genes ($p \approx 0$) from histone clusters 1–3, particularly in cluster 1, were down-regulated upon berberine treatment (Supplementary data 3). These genes are tightly associated with nucleosome assembly ($p < 0.05$) as well as telomere maintenance ($p < 0.05$) and, thus, are important for epigenetic regulation. Many gene sets associated with the polycomb complex 1/2 and DNA methylation, which regulate downstream pathways such as TNF-mediated apoptosis, NF-κB, Jak-STAT, and ERK/AKT, are also enriched (Supplementary data 4). Consistent with these findings, the expression of histone deacetylases HDAC8 and HDAC9 was also down-regulated. In contrast, the expression of histone acetyltransferase (HAT) P300/CBP was up-regulated upon berberine treatment. In addition, large portions of gene groups related to terms such as "HDAC (histone deacetylase)" or "berberine", found using the literature mining tool FABLE (www.fable.chop.edu), demonstrated many intersections with the genes identified in this study (Supplementary data 2C), thereby strongly suggesting a non-random association among the genes.

3.2. Reverse-docking for berberine

To identify the putative molecular targets of berberine, we performed a reverse docking experiment to predict drug targets based on the docking of the 3D structure of berberine against a collection of 3D structures of known proteins (Li et al., 2006). This docking identified lysine-*N*-methyltransferase as a putative candidate targeted by berberine (Supplementary data 5).

3.3. Effects of berberine on epigenetic regulators

Because the above two analyses suggested the potential involvement of berberine in epigenetic regulation, we performed PCR

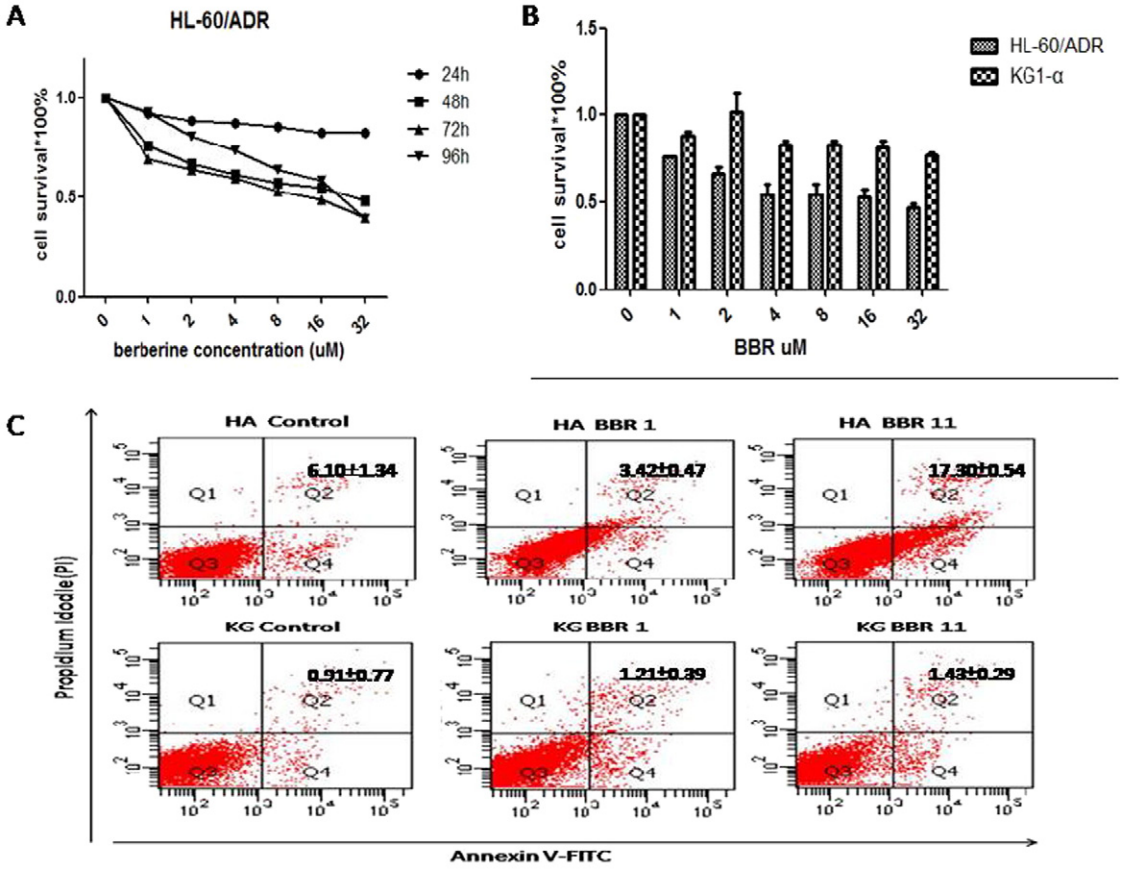

Fig. 1. BBR inhibits cell growth and induce apoptosis in HL-60/ADR and KG1-α cells. After exposed to different concentration BBR for 24, 48, 72, 96 h, HL-60/ADR cells (A) viability was measured by MTS assay. Viability of HL-60/ADR and KG1-α cells (B) after exposed to BBR for 48 h were compared. Cells were stained with Annexin V-FITC/PI and subjected to flow cytometry to determine apoptotic cells, including early apoptotic cells (lower right quadrant) and late apoptotic cells (upper right quadrant) (C); HA means HL-60/ADR cells, and KG means KG1-α cells.

experiments to determine the involvement of genes that are known to be epigenetic regulators. The PCR array of the human epigenetic chromatin modification enzymes allows the profiling of the expression of 84 key genes that encode enzymes, which are known or predicted to modify genomic DNA and histone substrates to regulate chromatin accessibility and gene expression. We quantified the expression of these genes with at least 1.5-fold change in U266 cells with or without berberine. As summarized in Table 1, several key observations were made: (1) Five histone acetylase levels represented by EP300 and CREBBP were up-regulated, whereas at least two histone deacetylase levels, HDAC2 and HDAC8, were reduced; (2) the lysine (K)-specific demethylase family was fairly active, among which KDM1 targets H3K9me2/1 and H3K4me2/1, KDM4 demethylates both H3K9me3/2 and H3K36me3/2, HDM5 is specific for H3K4me3/2 and KDM6 targets H3K27me3/2; and (3) the SET domain-containing histone methyltransferase family was also fairly active. SET1 and SET7 methylate H3K4, whereas SETB methylates H3K9. Both SET2 and SET3 methylate H3K36. SET6 mono-methylates K310 of the RelA subunit of the NF-κB complex. SETD8 mono-methylates H3K20, and SMYD3 di- and tri-methylates H3K4. However, there is a discrepancy for SMYD3 expression between microarray and PCR array data. After confirming the results using RT-qPCR, we found that SMYD3 was down-regulated and not up-regulated by berberine; (4) DNA methyltransferases, which are responsible for de novo methylation (DNMT3) or maintenance of methylated CpG (DNMT1), were also down-regulated (Table 1).

3.4. Berberine induces cytotoxicity and apoptosis in HL-60/ADR and KG1-α cells

We examined the effect of berberine on the cell viability using the MTS assay in AML lines (Fig.1A). HL-60/ADR cells were treated with different concentrations of BBR for 24, 48, 72 and 96 h. Based on the growth inhibition assay, BBR inhibit cell growth obviously in HL-60/ADR cells after 48 h exposed. When KG1-α cells were exposed to same concentrations of BBR, the survival rate was higher than HL-60/ADR cells (Fig.1B). HL-60/ADR cells were treated with 1 μM and 11 μM BBR, for 48 h, and stained with Annexin V-FITC/PI. Apoptosis was determined by flow cytometry, including early apoptotic cells (Annexin V-FITC positive, PI negative) and late apoptotic cells (Annexin V-FITC and PI positive). Berberine exhibited weekly cytotoxic activity in the KG1-α cells (Fig.1C).

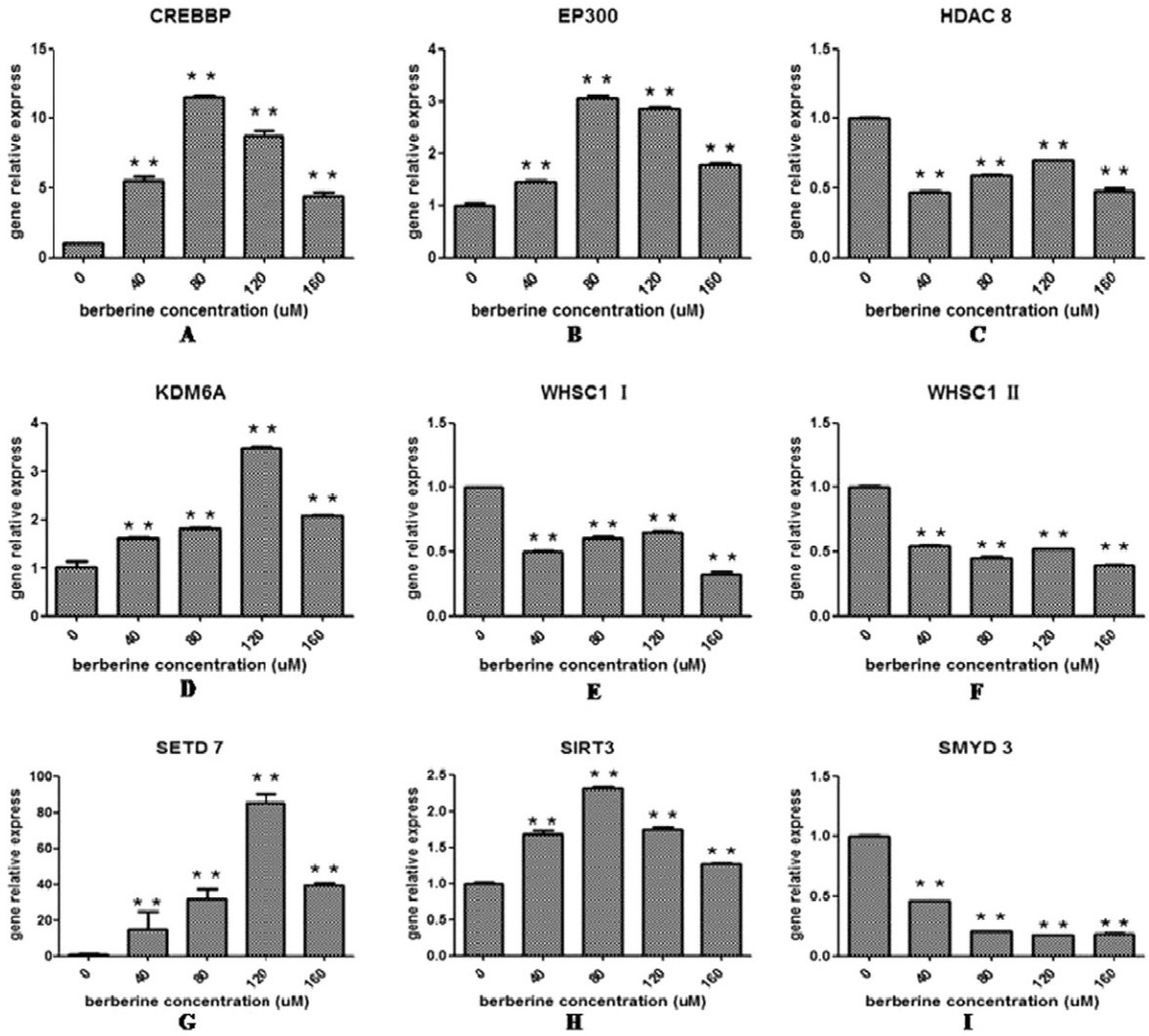

Fig. 2. Quantification of gene expression by Real-time PCR. Human multiply myeloma U266 cells were incubated with 0, 40, 80, 120, 160 μM of berberine for 48 h prior to extraction of total RNAs. PCR reaction was done as described in Materials and methods section. Relative mRNA levels of CREBBP (A), EP300 (B), HDAC8 (C), KDM6A (D), WHSC1I (E), WHSC1II (F), SETD7 (G), SIRT3 (H), SMYD3 (I) were measured respectively. The real-time PCR results were standardized against GAPDH, and the relative ratios were calculated. **: significant difference observed for $p < 0.01$.

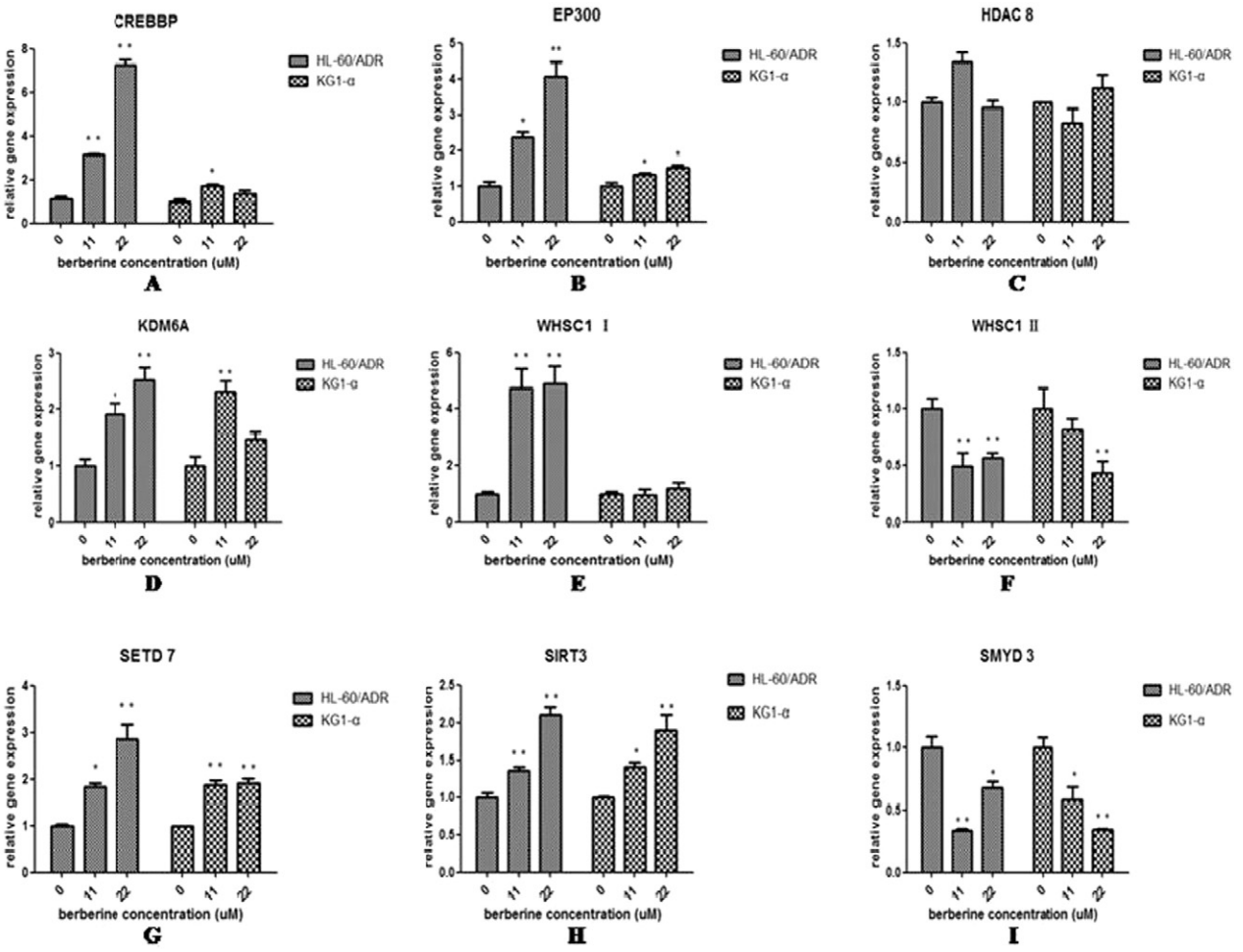

Fig. 3. Quantification of gene expression by Real-time PCR. HL-60/ADR and KG1-α cells were incubated with 0, 11, 22 μM of berberine for 48 h prior to extraction of total RNAs. Relative mRNA levels of CREBBP (A), EP300 (B), HDAC8 (C), KDM6A (D), WHSC1I (E), WHSC1II (F), SETD7 (G), SIRT3 (H), SMYD3 (I) were measured respectively. The real-time PCR results were standardized against GAPDH, and the relative ratios were calculated. **: significant difference observed for $p < 0.01$, *: significant difference observed for $p < 0.05$.

3.5. Real-time PCR confirmation of PCR array results

Confirmation of the PCR array experiment was performed using standard quantitative real-time PCR (qRT-PCR). We selected genes to further examine based on the PCR array results, as well as their potential function on epigenetic changes in previous reports. To confirm the universality of epigenetic regulation of berberine in hematological malignancy, we also confirmed gene expression using qRT-PCR in AML cell lines, HL-60/ADR and KG1-α.

The mRNA expression of CREBBP, EP300 and SIRT3, all of which are members of class III histone acetyltransferases (HDACs), was significantly higher in the berberine-treated group compared to the control group, whereas class I HDACs, which are represented by HDAC8, showed lower mRNA expression in the berberine-treated group (Fig. 2). The expression of histone methyltransferase and demethylase mRNA were also measured. Our data showed that SETD7 and KDM6A were up-regulated, while WHSC1I, WHSC1II and SMYD3 were down-regulated, upon treatment. These results were consistent with the results obtained from the qRT-PCR and PCR array, except for the high expression of SMYD3, which was down-regulated in both the microarray and qRT-PCR experiments, presumably due to an experimental error in the PCR array. Similar results were observed in HL-60/ADR and KG1-α cells. However, the only difference was the expression of WHSC1I, which was higher after berberine treatment, and there was no difference in HDAC8 (Fig. 3).

3.6. Berberine affects the level of H3K4me2, H3K4me3, H3K27me3, and H3K36me3 proteins

Similar to other post-translational modifications, methylation of various lysine (K) residues on histones, particularly on histone 3 (H3) could

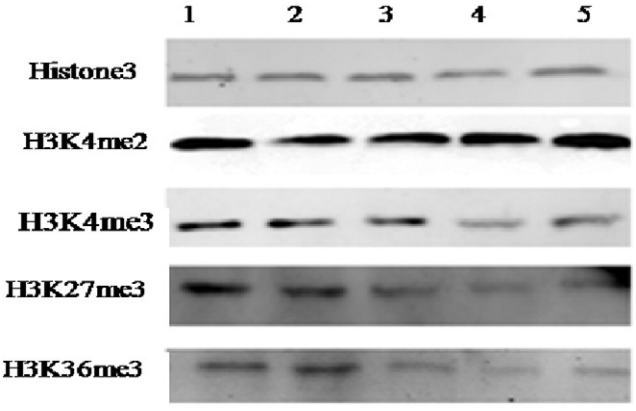

Fig. 4. Semi-quantitative assessment of H3K4me2, H3K4me3, H3K27me3, H3K36me3 protein levels by Western blot analysis. Lanes 1–5: cells were incubated with 0, 20, 40, 80, 120 μM of berberine for 24 h. Protein samples were prepared and histone modifications were quantified as described in Materials and methods section. Level detected by anti-Histone3 (top) was used as internal reference for normalization. The proteins Histone3, H3K4me2, H3K4me3, H3K27me3 and H3K36me3 were the expected size of 17KDa.

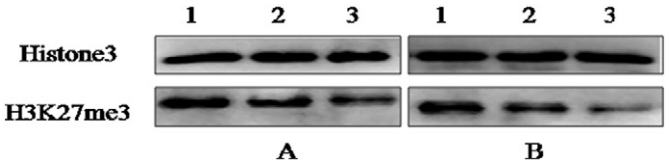

Fig. 5. Semi-quantitative assessment of H3K27me3 protein levels by Western blot analysis. Picture A and B correspond to HL-60/ADR cells and KG1-α cells, respectively. Lanes 1–3: cells were incubated with 0, 11, 22 μM of berberine for 48 h. Protein samples were prepared and histone modifications were quantified as described in Materials and methods section. Level detected by anti-Histone3 (top) was used as internal reference for normalization.

affect the chromosomes' degree of openness and transcriptional activity. H3K27 and H3K9 are often associated with gene silencing, whereas H3K4 and H3K36 are often linked with the active transcribing region (Bartova et al., 2008). On the basis of the altered expression profiles of genes participating in methylation or demethylation by berberine, we examined whether berberine treatment affects the methylation status of histone 3 protein in U266 cells. Thus, the protein levels of methylated histone H3K4me2, H3K4me3, H3K27me3, and H3K36me3 were measured semi-quantitatively using western blot analysis. Most of these protein expressions trended lower as concentrations of berberine increased, with the exception of H3K4me2 (Fig. 4). In HL-60/ADR and KG1-α cells, the protein levels of H3K27me3 were reduced after treatment of berberine (Fig. 5).

4. Discussion

The epigenetic-regulating effects of berberine have not yet been studied. On the basis of the bioinformatics analyses, predictions and experimental validations, our present study demonstrates the potential of berberine as an epigenetic modulator. Many gene sets associated with polycomb complex 1/2 epigenetic regulation were enriched by berberine (Supplementary data 4). Our data supported the hypothesis (Fig. 6) that berberine interacts with master regulators or interferes with the formation of regulatory complexes in chromatin remodeling, which is responsible for the control of downstream bioactivities, such as cell cycle, apoptosis, and mechanisms related to metabolic disorders, and immune response. Histone acetylation is an important post-translational modification in the regulation of gene expression. CBP and p300 proteins, which exhibit acetylase activity, are transcriptional co-activators and hematopoietic tumor suppressors. Their loss may result in aberrant differentiation or directly affect acetylation targets, such as p53 and TCF(Kung et al., 2000). However, HDACs are more often related to gene silencing. HDAC8 is overexpressed in childhood acute lymphoblastic leukemia (ALL) compared with normal bone marrow cells (Moreno et al., 2010). HDAC8 may also mediate invasion and up-regulate MMP-9 expression in breast cancer (Park et al., 2011). Here, we observed that berberine up-regulated the expression of CBP, EP300 and SIRT3 and down-regulated the expression of HDAC8 in U266 cells. In HL-60/ADR and KG1-α cells, CBP, EP300, SIRT3 were also up-regulated, but HDAC8 did not have significant changes and was WHSC1l up-regulated on the contrary. Interestingly, SIRT3 can function as a context-dependent tumor-specific promoter or suppressor. For example, SIRT3 suppresses tumorigenesis and induced cell death in colorectal carcinoma, osteosarcoma, leukemia, and breast cancer, as reviewed by Turki Y (Alhazzazi et al., 2011). Our study suggested that berberine induces histone acetylation and may result in the activation of genes that are responsible for growth suppression and apoptosis in U266, HL-60/ADR and KG1-α cells.

Wolf–Hirschhorn syndrome candidate gene 1 (WHSC1; also known as NSD2 and MMSET) has been reported to methylate H3K36, as well as H4K20, H3K4 and H3K27 proteins (Wagner and Carpenter, 2012). WHSC1-mediated disruption of H3K36me2 protein organization may promote oncogenic programming in many cell types, particularly in multiple myeloma. In myeloma cells, the expression of WHSC1 is associated with global H3K36me2 protein levels and the cell proliferation

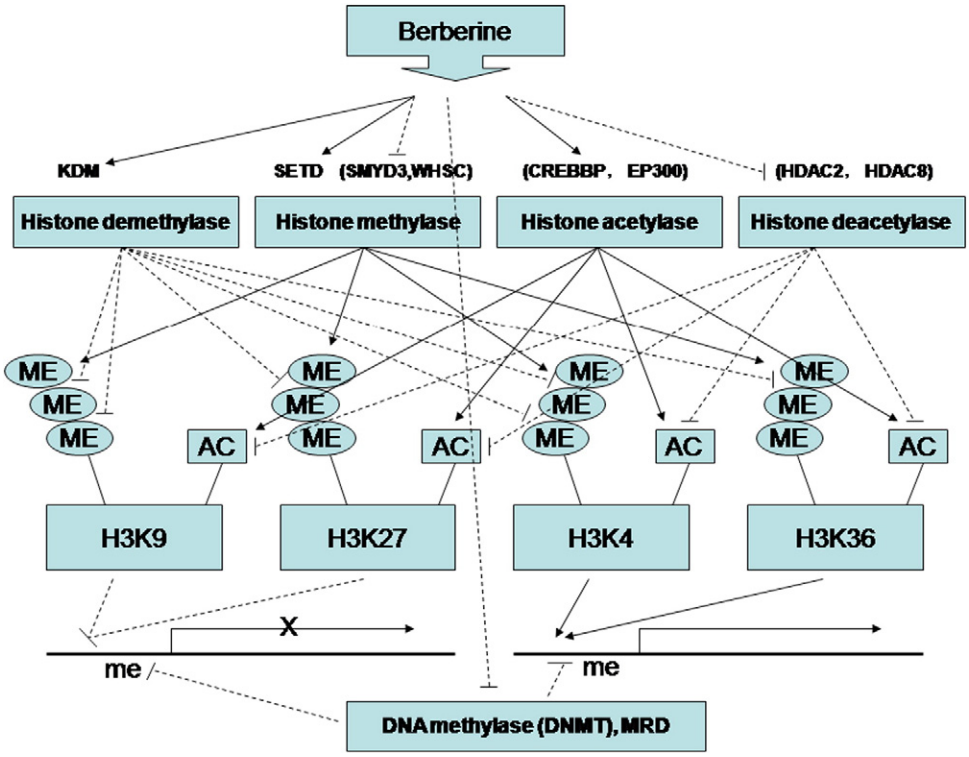

Fig. 6. A hypothetical model of epigenetic regulation by berberine on epigenetic chromatin modification enzymes. From this study, we propose a model in which berberine modulates protein levels of methylation of H3K4, H3K27, H3K36 by altering levels of several families of specific modification enzymes, and thus turning on/off downstream targets/pathways. Dash line: inhibition; solid line: activation.

rate (Kuo et al., 2011; Martinez-Garcia et al., 2011). Loss of WHSC1 function decreases H3K36me2 and H3K36me3 levels, which is accompanied by a concomitant decrease in the global levels of H3K27me2 and H3K27me3 proteins (Wagner and Carpenter, 2012). Our results showed that berberine reduced the levels of H3K27me3 and H3K36me3 proteins in U266 cells, reduced the mRNA expression of WHSC1, and increased the mRNA expression of KDM6A, whose product acts as a histone demethylase specific for H3K27me3/2 proteins. Bi-allelic somatic mutations and down-regulation of KDM6A have been described in multiple tumor types, and low KDM6A activity is correlated with poor prognosis (Lim et al., 2010). Recently, it was hypothesized that an increase in H3K27 methylation, via either WHSC1 overactivity or loss of KDM6A function, is advantageous to myeloma cell proliferation (Kim et al., 2008; Smith et al., 2010). The present study demonstrated the effects of berberine on the suppression of cell growth and promotion of cell differentiation by decreasing H3K36me3 protein levels via the down-regulation of WHSC1 and/or up-regulation of KDM6A expression in U266, HL-60/ADR and KG1-α cells.

Methylation of lysine 4 (K4) on H3 is associated with euchromatic regions and H3K4 tri-methylation (H3K4me3) in general, which marks actively transcribed genes. However, H3K4me3 and H3K27me3 proteins often readily form the so-called "bivalent" histone marks for silenced genes for transcription. H3K4me2 and H3K4me3 levels are increased in many cancers (Chen et al., 2011; Ellinger et al., 2010). Jugova et al. (2011) observed that compared with leukemia cells, H3K4 methylation in MM cells is more susceptible to selective inhibition of HDACs and DNMTs. Histone methyltransferase SMYD3, a histone H3K4 specific di- and tri-methyltransferase, has been reported to repress tumor suppressors while promoting oncogenes (e.g., *N*-myc, CrkL, Wnt10b, RIZ and hTERT) and genes involved in the control of cell cycle (e.g., CCNG1 and CDK2) or signal transduction (e.g., STAT1, MAP3K11 and PIK3CB) (Cock-Rada et al., 2012; Foreman et al., 2011; Hamamoto et al., 2004; Ren et al., 2011). Although the SMYD3 expression observed in our PCR array experiment appeared aberrant, we confirmed a consistent reduction in SMYD3 mRNA expression in an independent PCR experiment (Fig. 2), which was consistent with findings of decreased levels of H3K4me3 protein after berberine treatment. These findings suggest that berberine potentially reduces the levels of H3K4me3 protein by down-regulating SMYD3 expression.

5. Conclusion

We conclude that the antitumor effects of berberine may result from the modulation of key epigenetic regulators. This offers a novel explanation of how berberine initiates so many therapeutic activities in the clinic. Although our bioinformatics and experimental study supports this working hypothesis, additional experiments should be performed to confirm these findings (e.g., to dissect the interplay between berberine and its target(s) and the downstream signaling cascade).

Corresponding authors' contributions

Shuang Liang, Fanyi Meng and Zhigang Lu conceptualized and designed the study, and drafted the paper.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

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